Effect of Replacing Alfalfa Silage With High Moisture Corn on Ruminal Protein Synthesis Estimated From Urinary Excretion of Purine Derivatives

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Introduction

More than half of the protein in alfalfa silage (**AS**), one of the most important forages fed to dairy cows in the U.S., gets broken down to NPN in the silo. Utilization of AS NPN is stimulated by high energy feeds, such as high moisture ear corn (**HMC**), by increasing microbial protein formation in the rumen. However, over-feeding of high energy feeds can result in adverse effects in the cow's rumen. Thus, it is important to determine the amount of HMC that can be fed to obtain the maximum utilization of AS NPN without impairing animal performance. Urinary excretion of purine derivatives (**PD**) has been used to measure microbial protein synthesis in the rumen. This trial quantified ruminal microbial protein from urinary PD excretion in lactating cows fed alfalfa silage that was supplemented with varying levels of HMC-based concentrate.

Materials and Methods

Twenty-four multiparous Holstein cows, including 8 with ruminal cannulae, were assigned to six 4 X 4 Latin squares with 3-wk periods (total 12 wk). Four diets (see Table 1 of the companion Research Summary), fed as TMR, contained (DM basis): 80, 65, 50 or 35% AS as the sole forage plus 20, 35, 50 or 65% concentrate. The concentrate consisted mainly of ground HMC; soybean meal and urea were added to make diets equal in CP and NPN (43% of total N). Allantoin was determined in milk samples taken on d-12 and d-19 of each period. Ruminal contents were taken at 0, 1, 2, 3, 4 and 6 h postfeeding on d-15 of each period and analyzed for pH, NH_3, total free AA and VFA. Total, 24-h urine collections were made using indwelling bladder catheters on d-19 of each period; daily urinary excretion of allantoin, uric acid, creatinine and urea was determined. Total PD excretion was the sum of urinary allantoin and uric acid excretion plus milk allantoin excretion. Ruminal microbial protein synthesis was computed using these equations (Chen and Gomes 1992): Total absorption of microbial purines (mmol/d) = (total PD excretion - 0.385 $BW^{0.75}$)/0.85, where 0.385/$BW^{0.75}$ is endogenous PD excretion (mmol/d) and 0.85 is purine absorptive efficiency; and 2) net synthesis of microbial N (g/d) = (purine absorption x 70) / (0.116 x 0.83 x 1000), where 70 is the N content of purines (mg N/mmol), 0.116 is the ratio of purine-N: total-N in mixed rumen microbes and 0.83 is the assumed digestibility of microbial purines. Other trial details are described in the companion Research Summary.

Results and Discussion

Daily urine volumes were 48.3, 48.4, 41.8, and 31.5 kg/d for diets containing, respectively, 20, 35, 50, and 65% concentrate. Urine output decreased linearly with increasing concentrate (Table 3) probably due to the decrease in dietary K, lowest to highest concentrate level, from 2.12 to 1.23% K (DM basis). Replacing AS, the major dietary source of K, with HMC and soybean meal accounted for this decrease. Daily metabolite excretions are summarized in Table 1. As expected, urinary creatinine excretion was a constant function of BW. Urinary excretion of allantoin and uric acid, and total PD excretion, were higher in cows fed 50 than 20% concentrate. All three traits were influenced quadratically by concentrate level, with maxima averaging about 48% dietary concentrate (Table 3). Daily urinary excretion of allantoin varied from 369 to 535 mmol/d. The proportion of urinary allantoin in total PD excretion was constant, ranging from 90.2 to 90.7%. Milk allantoin secretion increased linearly with concentrate ranging from 18.7 to 28.6 mmol/d and accounted for 4.2 to 5.7% of the total PD excretion. Urea N excretion declined from a maximum of 342 to 239 g/d with increasing concentrate, indicating better NPN utilization with greater energy intake. Maximum urea N excretion was predicted to occur at 36% concentrate (Table 3). Total PD excretion ranged from 423 to 613 mmol/d and was highest for cows fed 50% concentrate (Table 1). Ruminal microbial N yields, computed from PD excretion using the equations of Chen and Gomes (1992), ranged from 278 to 419 g/d (Table 1). Of course, maximum microbial yield also was predicted to occur at 48% concentrate (Table 3). This was consistent with the maximum DM intake estimated at 51% dietary concentrate (companion Research Summary). Microbial N yields in the rumen also were computed using dietary NE_L, calculated from apparent OM digestibilities, and DM intakes using the NRC equation: Microbial N = - 30.93 + 11.45 x NE_L (Mcal/d). Estimates of 280, 356, 395 and 420 g/d were obtained for, respectively, diets containing 20, 35, 50 and 65% concentrate. Subtracting these from estimates computed with PD excretion yielded differences of -2, -23, +24 and -85 g/d, respectively. The large difference only at the highest level of concentrate is interesting and may be attributed to reduced pH and other changes in the environment of the rumen that may depress net yield of microbial protein. Mean ruminal pH over the 6 h after feeding declined from 6.5 (20% concentrate) to 6.1 on both 50 and 65% concentrate (Table 2). That microbial N yield was 20% lower (335 versus 419 g/d) on 65 than on 50% dietary concentrate suggested that factors in addition to low ruminal pH contributed to depressed microbial protein formation on the highest concentrate diet. Overall, microbial N yields estimated from total PD excretion were consistent with observed yield of milk and milk components on the same diets.

Mean ruminal pH of cows fed 20% concentrate was higher than at 50 and 65% dietary concentrate (Table 2) and remained higher than on the other three diets over the 6 h after feeding (Fig. 1). Ruminal pH on 35% dietary concentrate was intermediate (Table 2) and did not change due to time after feeding (Fig. 1). Reductions of ruminal pH of short duration likely cause only moderate, transient depression in fiber digestion; however, pH reductions for longer periods may cause washout of the ruminal organisms associated with fiber digestion and severely reduce fiber and OM digestion and microbial protein yield. Ruminal pH after feeding fell below 6 at only one time (6-h after feeding) on 50% concentrate but was below pH 6 at three time-points on 65% concentrate (Fig. 1). Ruminal NH_3 was very high at all times after feeding (Fig. 2) and there were no differences among diets in mean concentration (Table 2). High ruminal NH_3 is not surprising at dietary CP levels of 19.5 to 20.1%, with 43% of the CP equivalent coming from NPN. There were differences in total AA concentrations after feeding (Fig. 3); overall, total AA tended to be higher on the 65% concentrate diet (Table 2), the diet that contained the most soybean meal. Peak ruminal NH_3 (Fig. 2) and total free AA concentrations (Fig. 3) for all diets occurred 1-h postfeeding. Ruminal total VFA were unaffected by diet (Table 2). Molar proportions of acetate decreased linearly and propionate increased linearly

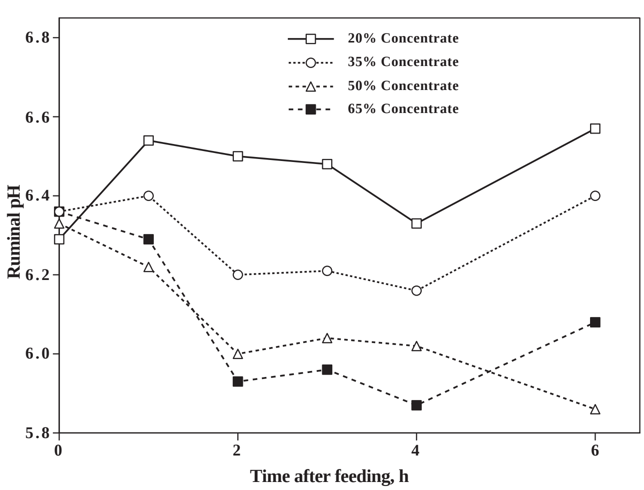

Figure 1. Mean hourly pH in the rumens of cows fed diets with all forage from alfalfa silage and 20, 35, 50 or 65% of dietary DM as a concentrate mix based on ground high moisture ear corn.

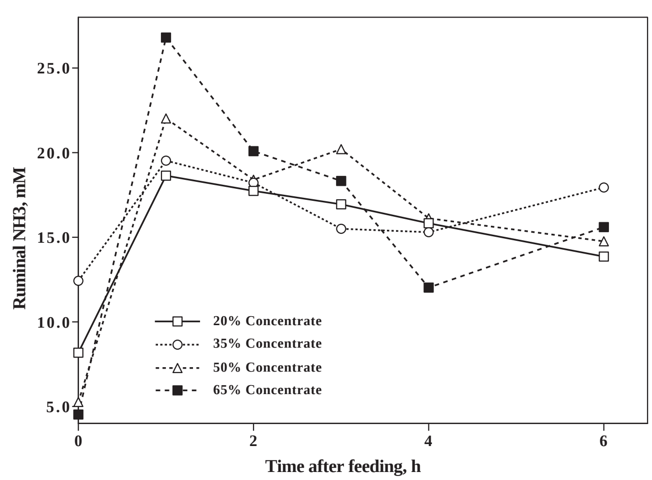

Figure 2. Mean hourly NH_3 concentration in the rumens of cows fed diets with all forage from alfalfa silage and 20, 35, 50 or 65% of dietary DM as a concentrate mix based on ground high moisture ear corn.

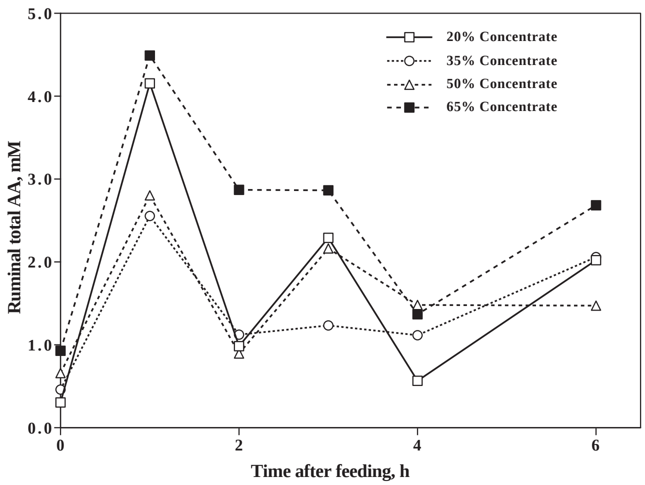

Figure 3. Mean hourly total AA concentration in the rumens of cows fed diets with all forage from alfalfa silage and 20, 35, 50 or 65% of dietary DM as a concentrate mix based on ground high moisture ear corn.

with decreased AS and increased concentrate in the diet; acetate: propionate ratio also declined linearly, from 3.58 to 2.00, with increased dietary concentrate (Table 2). These changes are typical when more nonstructural carbohydrate and less fiber is fermented in the rumen. Molar proportions of isobutyrate and isovalerate plus 2-methylbutyrate declined linearly with decreasing AS in the diet (Table 2). Branched-chain VFA are formed from microbial catabolism of branched-chain AA and their concentrations would be directly related to the intake of free AA and peptides in AS NPN.

Summary and Conclusion

Purine derivative excretions, measured in 24-h total urine collections, gave satisfactory estimates of microbial N yields in lactating cows fed varying levels of dietary concentrate to replace AS. Maximum yield of microbial N was observed at 48% dietary concentrate, suggesting that this was the optimum feeding level for HMC-based concentrate to maximize utilization of the NPN in AS.

Reference

Chen, B. and M.J. Gomes. 1992. Int. Feed Res. Unit, Occasional Publ., Rowett Res. Inst., Aberdeen, U.K. pp. 75-82.

Table 1. Effect of replacing dietary alfalfa silage with concentrate on daily excretion of milk allantoin and urinary creatinine, and of allantoin, uric acid and urea N measured in total urine collections and on microbial N synthesized in the rumen as estimated from total excretion of purine derivatives.[1]

	Dietary concentrate (% of DM)						
Item	20	35	50	65	SEM	L	Q
Milk allantoin, mmol/d	18.7[d]	22.7[c]	25.9[b]	28.6[a]	0.5	0.001	0.195
Total urine collection							
Creatinine, mg/kg BW/d	28.6	28.4	30.0	28.9	0.5	0.209	0.281
Allantoin, mmol/d	369.1[b]	435.2[ab]	534.8[a]	428.9[ab]	31.1	0.005	0.011
Uric acid, mmol/d	35.5[b]	43.4[ab]	52.4[a]	42.3[ab]	3.7	0.009	0.017
PD[2], mmol/d	423.3[b]	501.5[ab]	613.2[a]	499.8[ab]	34.1	0.004	0.009
Allantoin, % of PD	90.7	90.2	90.5	90.6	0.5	0.454	0.462
Microbial N, g/d	278.4[b]	336.0[ab]	419.3[a]	335.1[ab]	25.3	0.004	0.009
Urea N, g/d	303.7[b]	342.0[a]	307.9[b]	238.7[c]	7.5	< 0.001	< 0.001

[a,b,c]Means in rows with different superscripts differ ($P < 0.05$).
[1]L = Probability of linear effect, PD = total purine derivatives (allantoin + uric acid), Q = probability of quadratic effect.
[2]Including milk allantoin excretion.

Table 2. Effect of replacing dietary alfalfa silage with concentrate on ruminal pH, ruminal concentration of NH_3, total AA and total VFA, and on molar proportions of ruminal VFA.[1]

	Dietary concentrate (% of DM)						
Item	20	35	50	65	SEM	L	Q
pH	6.51[a]	6.29[ab]	6.08[b]	6.08[b]	0.11	0.001	0.195
NH_3, m*M*	15.20	16.49	16.13	16.23	2.32	0.005	0.011
Total AA, m*M*	1.72[ab]	1.42[b]	1.58[ab]	2.53[a]	0.61	0.009	0.017
Total VFA, m*M*	114.1	129.5	133.0	130.0	5.0	0.089	0.074
Molar proportion, mol/100 mol of total VFA							
Acetate (A)	64.4[a]	62.7[ab]	58.5[b]	52.7[c]	1.2	< 0.001	0.190
Propionate (P)	18.2[b]	19.1[b]	22.5[b]	28.6[a]	1.5	0.001	0.093
A: P ratio	3.58[a]	3.30[ab]	2.79[b]	2.00[c]	0.18	< 0.001	0.146
Butyrate	11.8	12.5	14.0	14.0	0.5	0.092	0.190
Isobutyrate	1.42[a]	1.38[a]	1.15[b]	0.97[c]	0.04	< 0.001	0.482
Isovalerate + 2-methylbutyrate	1.93[a]	2.04[a]	1.65[a]	1.20[b]	0.10	0.003	0.996
Valerate	2.20	2.28	2.23	2.47	0.14	0.674	0.072

[a,b]Means in rows with different superscripts differ ($P < 0.05$).

[1]L = Probability of linear effect, Q = probability of quadratic effect.

Table 3. Significant linear and quadratic regressions on dietary concentrate level.[1]

Variable (Y)	Type	Equation	$(R^2)^2$	Maximum[3]
Total urinary excretion				
Urine volume (kg/d)	Linear	$Y = 58.6 – 0.373\ C$	0.795	. . .
Allantoin (mmol/d)	Quadratic	$Y = 66.3 + 18.3\ C – 0.192\ C^2$	0.402	47.6%
Uric acid (mmol/d)	Quadratic	$Y = 3.80 + 1.99\ C – 0.021\ C^2$	0.396	47.5%
PD (mmol/d)	Quadratic	$Y = 82.8 + 20.6\ C – 0.215\ C^2$	0.411	48.0%
Urea-N (g/d)	Quadratic	$Y = 182 + 8.74\ C – 0.120\ C^2$	0.816	36.4%
Microbial N (g/d)	Quadratic	$Y = 26.0 + 15.3C – 0.159\ C^2$	0.413	48.1%
Ruminal VFA proportion				
Acetate (A)	Linear	$Y = 70.7 – 0.262\ C$	0.842	. . .
Propionate (P)	Linear	$Y = 12.3 + 0.231\ C$	0.782	. . .
A: P	Linear	$Y = 4.41 – 0.035\ C$	0.820	. . .
Isobutyrate	Linear	$Y = 1.68 – 0.010\ C$	0.879	. . .
Isovalerate + 2-methylbutyrate	Linear	$Y = 2.44 – 0.017\ C$	0.758	. . .

[1]C = Dietary concentrate (% of DM), and PD = total purine derivatives (allantoin + uric acid).

[2]Coefficient of determination.

[3]Dietary concentrate content (% of DM) at maximum determined by taking first-derivative of quadratic equations, where significant.